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An assessment of age determination in fossil fish: the case of the opercula in the Mesozoic actinopterygian *Saurichthys*

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An assessment of age determination in fossil fish: the case of the opercula in the Mesozoic actinopterygian *Saurichthys*

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Abstract Analyzing and interpreting life history data (e.g., aging and longevity, age at sexual maturity) are fundamental in many paleontological studies. In the case of extant and fossil fishes, otoliths, and to a lesser degree other incrementally growing hard tissue structures such as scales and bones, have been utilized to reveal these data. We investigated the microanatomy and internal microstructure of opercula of *Saurichthys*, one of the most easily recognized and globally distributed fishes in the Triassic, to elucidate whether these prominent skull bones provide reliable age estimates. Opercula, and where the subopercula is present, of several outgroup taxa were sectioned to provide a phylogenetic framework for the study. The external protrusions and ridges or internal concentric bands or wrinkles are not related to internal age-related bone tissue structures such as annuli and growth zones, but are instead purely ornamental in the case of the former and probably structural/taphonomic in case of the latter. Opercular morphogenesis of *Saurichthys* opercula differs from that of the extant outgroups examined in that they show rostro-medial to caudo-lateral extending growth increments instead of ones that extend sub-parallel to the opercular bone surfaces. Individual age data could thus not be reliably extracted from those opercula. Furthermore, an odontode-like complex was not encountered in *Saurichthys* opercula, but a few specimens show a peculiar, weakly

birefringent thin tissue layer of unknown origin filling the valleys between the external ornamental ridges. Although all *Saurichthys* opercula showed growth marks, these could not be counted to produce reliable individual age data, whereas bones with a more concentric cross section, such as the ceratohyal, appear better suited for this purpose.

Keywords Bone histology · Opercle · Sclerochronology · Triassic · Predatory fish · Actinopterygii

Introduction

Vertebrate hard tissues such as bones and teeth are known to record life history data throughout an animal's ontogeny and can be used for skeletochronology (also known as sclerochronology; see for example Castanet et al. 1993). In fishes, virtually all hard tissue structures such as skull and girdle bones, opercula, scales, fin rays, fin spines and vertebrae have been used for age estimation, but the ones yielding the most accurate data are in most cases the otoliths (e.g., Panfili et al. 2002). The identification of clear growth lines in all of these hard tissues have to follow careful protocols and visualization procedures which are sensitive to artifacts, and most skeletal elements sectioned besides otoliths might, in some cases substantially, underestimate the age on an individual (e.g., Cooley and Franzin 1995; Dan 1980; Jackson et al. 2007; Khan and Khan 2009; Kimmel et al. 2010; Ma et al. 2010; Perry and Casselman 2012; Phelps et al. 2007; Sipe and Chittenden 2001). Validation of age estimations has been attempted in various extant fish species, using various methods including capture–recapture studies by means of tagging, dye injection or radioisotope and elemental marking (e.g., Babaluk and Campbell 1987; Beamish and McFarlane 1993; Bruch et al.

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2009; Campana 2001; Panfili et al. 2002; Rossiter et al. 1995).

Of the hard tissues mentioned above, opercula are often used in fisheries studies (reviewed in Campana 2001; Panfili et al. 2002), mainly because they are easily identifiable bones, as well as handled and studied for age estimation without difficulties. The opercula generally grow appositionally, with increments of newly added bone showing up as dark and light bands, as in the manner of tree rings (e.g., Casselman 1987; Devries and Frie 1996). Age assessment based on opercular bones relies on the recognition of these periodic growth increments (annual marks, rings or annuli for yearly growth) that can be discerned and counted. Age estimates based on opercula are reported to reach the accuracy provided by otoliths in specific cases; for example in perch *Perca flavescens* and *P. fluviatilis* (Bardach 1955; Le Cren 1947), in white bass *Morone chrysops* (Soupir et al. 1997), in catfish *Tachysurus tenuispinis* (Dan 1980), in goldeye *Hiodon alosoides* (Donald et al. 1992), in lake trout (Sharp and Bernard 1988), in walleye *Sander vitreus* (= *Stizostedion vitreum*; Babaluk and Campbell 1987; Campbell and Babaluk 1979), and in carp *Cyprinus carpio* (McConnell 1952).

Opercula are very prominent skull bones in most extant and extinct ray-finned fishes (Actinopterygii), a group representing approximately half of all living species of vertebrates (Gardiner et al. 2005; Helfman et al. 2009). Opercula in many cases are species specific in shape. In fact, opercular morphometrics serves to distinguish, for example, species of *Saurichthys*, a basal actinopterygian, which was a globally widespread predator during the Triassic period (Rieppel 1985, 1992; Wilson et al. 2013).

Saurichthys is nested in the clade Saurichthyidae, comprising either two (Romano et al. 2012) or three additional genera (Wu et al. 2011), depending whether *Sinosaurichthys*, besides *Eosaurichthys* and *Saurorhynchus*, is a valid genus or not (Maxwell et al. 2013). *Saurichthys* displayed a considerable diversity with over 35 currently recognized species (Mutter et al. 2008; Romano et al. 2012). The species from the Middle Triassic UNESCO world heritage site Monte San Giorgio, Ticino, are known from hundreds of specimens in a controlled stratigraphical and palaeoecological context (Maxwell et al. 2013; Renesto and Stockar 2009; Rieppel 1985, 1992). A second similar-aged fossil site in the Ducan area south of Davos, Switzerland (Furrer 2009) also contains exceptionally preserved *Saurichthys* material. *Saurichthys* offers thus the chance, in principle, to study demographic aspects of populations inhabiting different environments, with the ultimate goal being to understand the adaptive radiation of species flocks in this animal (Wilson et al. 2013). In addition, life history data of *Saurichthys*, species of which range from a few tens of centimeters to about

1.5 m in body length (e.g., Mutter et al. 2008), would increase our understanding of the importance of these predators at several levels in the ancient food webs of the Triassic oceans (Wu et al. 2011).

In *Saurichthys*, there is only a single large opercle bone present (Fig. 1a), which covers the animal's gill area. Circular markings on the surface of the opercula, interpreted as growth lines, have been reported for several species of *Saurichthys* including *S. curionii* (Griffith 1959; Rieppel 1985), *S. macrocephalus* (Rieppel 1985), *S. striolatus* (Griffith 1959), and *S. krambergeri* (Griffith 1962). Authors do not agree, however, whether this observation is possible on the external (Griffith 1959) or the internal surface (Rieppel 1985) and none provided exact counts of these lines. Furthermore, up to our knowledge, sectioning opercula for studying the internal microanatomy for growth patterns was never attempted in *Saurichthys*, and among extant fish, was reported only for a few species so far, including the longnose sucker *Catostomus catostomus* (Perry and Casselman 2012) and the shovelnose sturgeon *Scaphirhynchus platyrhynchus* (Jackson et al. 2007), yielding variable results.

It is not possible to study the whole fossil opercula in transmitted light, therefore, we provide here histological and surface microanatomical data on opercula of *Saurichthys* from the Middle Triassic of Monte San Giorgio, as well as on three outgroup taxa, to elucidate growth patterns and age structures. Three non-teleost actinopterygians, including the Nile bichir, the Siberian sturgeon and the spotted gar (Fig. 1b; based on Coates 1999; which is in accordance with newer works by Gardiner et al. 2005; Near et al. 2013), were used to provide an extant phylogenetic bracket (Witmer 1995) to interpret our fossil data.

The following questions are addressed herein: are growth marks preserved within *Saurichthys* opercular bone tissue and do they provide reliable age estimates? Does the external ornamentation or internal markings of *Saurichthys* opercula reflect the age of individuals? Are growth patterns of *Saurichthys* opercula comparable to those of other (extant) fish? Would bones with a cylindrical cross-sectional shaft area reveal life history data if opercula are not available for study?

Materials and methods

The materials used for the study include eight opercula of *Saurichthys* sp. from the Middle Triassic of Monte San Giorgio, collected during systematic excavations under direction of Bernhard Peyer and Emil Kuhn-Schnyder (Paläontologisches Institut und Museum, University of Zurich, PIMUZ) in the years 1930–1933 and 1956–1973. The material spans two formations (Besano Formation,

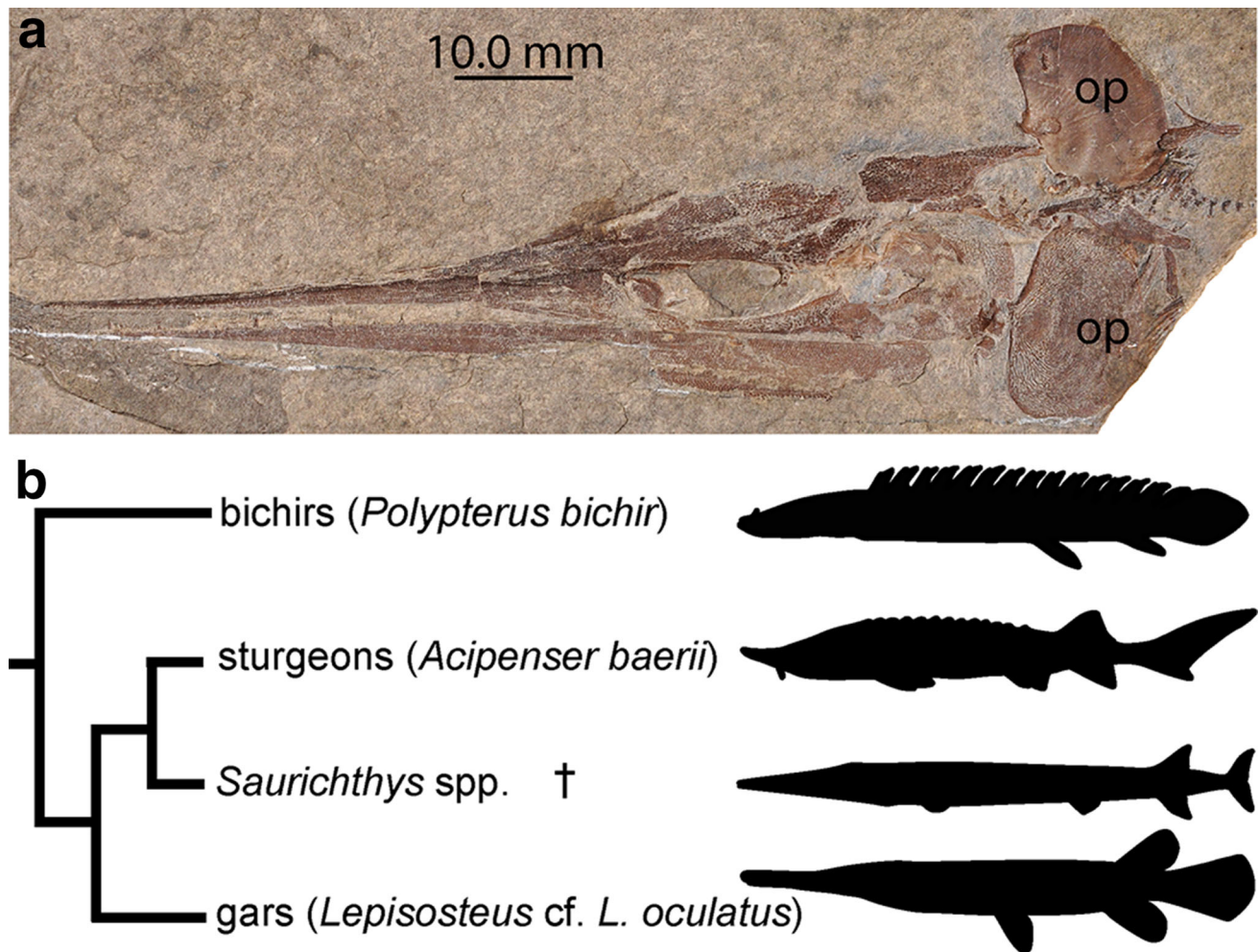


Fig. 1 The basal actinopterygian *Saurichthys*. **a** Unprepared skull of *Saurichthys curionii* (PIMUZ T 2718, mirror image) from the Lower Ladinian (Cassina Member, Meride Formation, Middle Triassic) of Monte San Giorgio, Switzerland. Note prominent oval opercula (*op*)

in internal (*top*) and external (*bottom*) view, the latter showing strong surface ornamentation; **b** phylogenetic hypothesis showing the position of *Saurichthys* in the framework of non-teleost Actinopterygii, following Gardiner et al. (2005) and Near et al. (2013)

Late Anisian, and Meride Formation, Early Ladinian). One of the eight opercula, together with a ceratohyal (46 mm in length, 2.7 mm shaft diameter), belong to a very large specimen (PIMUZ T 2138), estimated to be close to 1.5 m, in total body length (Fig. 3). Although the ceratohyal is usually not among the most frequently used elements in skeletochronological studies, this bone was chosen here because of its sub-circular shaft region. Opercular bones of extant taxa, including one Nile bichir (*Polypterus bichir*), five Siberian sturgeons (*Acipenser baerii*) comprising two age classes, and one spotted gar (*Lepisosteus* cf. *L. oculatus*), were sampled to provide a phylogenetic framework for the study (Table 1). High resolution images in natural light of one opercle (PIMUZ T 2566) and of the ceratohyal bone (PIMUZ T 2138) have been added as Electronic Supplementary Material ESM1 and ESM2, respectively.

In the case of the Nile bichir and the spotted gar, it is unclear if the animals were pet-trade/zoo animals or caught in the wild before coming to the collections of the Naturhistorisches Museum Basel (MMB) and the PIMUZ, respectively. In the case of the Siberian sturgeon, the specimens were provided by the aquaculture facility of the Tropenhaus Frutigen, Switzerland (www.tropenhaus-frutigen.ch). The specimens from the younger age class (about 3¾ years old at time of death) were reared under near outdoor conditions (continuous flow facility or “Durchlaufanlage”) with seasonal temperatures ranging from about 6 to 20 °C. The old animals, of the 6-year age class, were initially also reared under conditions with marked seasonal fluctuations (ca. 6–20 °C) and then under more stable temperate conditions (ca. 14–20 °C).

Table 1 Specimens used in the study

Taxon sampled	Specimen number	Elements sectioned	Age and locality data, additional notes
Saurichthyidae			
<i>Saurichthys</i> sp.	PIMUZ T 757a	Opercle (fossil specimen)	Upper Besano Formation, Late Anisian, Mirigioli (P. 902), Meride, Ticino, Switzerland
<i>Saurichthys</i> sp.	PIMUZ T 2138	Opercle and ceratohyal bone (fossil specimen)	Middle Besano Formation, Late Anisian; Cava Tre Fontane, Meride, Ticino, Switzerland
<i>Saurichthys</i> sp.	PIMUZ T 2566	Opercle (fossil specimen)	Middle Besano Formation, Late Anisian; Valle Stelle, Meride, Ticino, Switzerland
<i>Saurichthys</i> sp.	PIMUZ T 2641	Opercle (fossil specimen)	Cassina Member, Meride Formation, Early Ladinian; Cassina, Meride, Ticino, Switzerland
<i>Saurichthys</i> sp.	PIMUZ T 5501	Opercle (fossil specimen)	Cassina Member, Meride Formation, Early Ladinian; Cassina, Meride, Ticino, Switzerland
<i>Saurichthys</i> sp.	PIMUZ T 5736	Opercle (fossil specimen)	Cassina Member, Meride Formation, Early Ladinian; Cassina, Meride, Ticino, Switzerland
<i>Saurichthys</i> sp.	PIMUZ T 5740	Opercle (fossil specimen)	Cassina Member, Meride Formation, Early Ladinian; Cassina, Meride, Ticino, Switzerland
<i>Saurichthys</i> sp.	PIMUZ T 5788	Opercle (fossil specimen)	Cassina Member, Meride Formation, Early Ladinian; Cassina, Meride, Ticino, Switzerland
Acipenseridae			
<i>Acipenser baerii</i> (Siberian sturgeon)	PIMUZ A/I 4307; A/I 4308	Right subopercle (macerated specimens)	Extant, aquaculture raised in France/Switzerland (two female specimens, between 3 and 4 years old)
<i>Acipenser baerii</i> (Siberian sturgeon)	PIMUZ A/I 4309; A/I 4010; A/I 4011	Right subopercle (macerated specimens)	Extant, aquaculture raised in France/Switzerland (three female specimens, ca. 6 years old)
Lepisosteidae			
<i>Lepisosteus</i> cf. <i>L. oculatus</i> (Spotted gar)	PIMUZ A/I 4170	Right subopercle and opercle (macerated specimen)	Extant, USA (no exact locality data available); tentatively identified as <i>L. oculatus</i> based on narrow exposed anterodorsal subopercular process, straight articulation of opercle with dermohyal and rounded ventroposterior margin of subopercle)
Polypteridae			
<i>Polypterus bichir</i> (Nile bichir)	NMB 2793	Right subopercle and opercle (removal from wet specimen stored in alcohol)	Extant; standard length 54 cm, total length ca. 59 cm (caudal fin slightly damaged, <1 cm) (inheritance by R. Burckhardt in 1914)

After embedding in synthetic resin (Araldit XW396 and XW397), the bones of both fossil and extant species were sectioned with a diamond-studded saw blade through the center of ossification. The planes of sectioning extended either anteroposteriorly through the opercula or the subopercula or along the longest axis of the element (see Figs. 2, 3). The sectioned bones were ground using different SiC powders (SiC 500, SiC, 800), covered with a thin glass slip using UV glue (Panacol Vitralit 6127) and then studied in normal and cross-polarized light with a compound microscope Leica DM2500 M, equipped with a digital camera LEICA DFC420 C. Digital images were processed using Adobe Creative suit software CS6 (Photoshop and Illustrator).

The opercula and subopercula studied herein are paired bones pertaining to the cranial exoskeleton, dermal in origin and not preformed in cartilage, whereas the

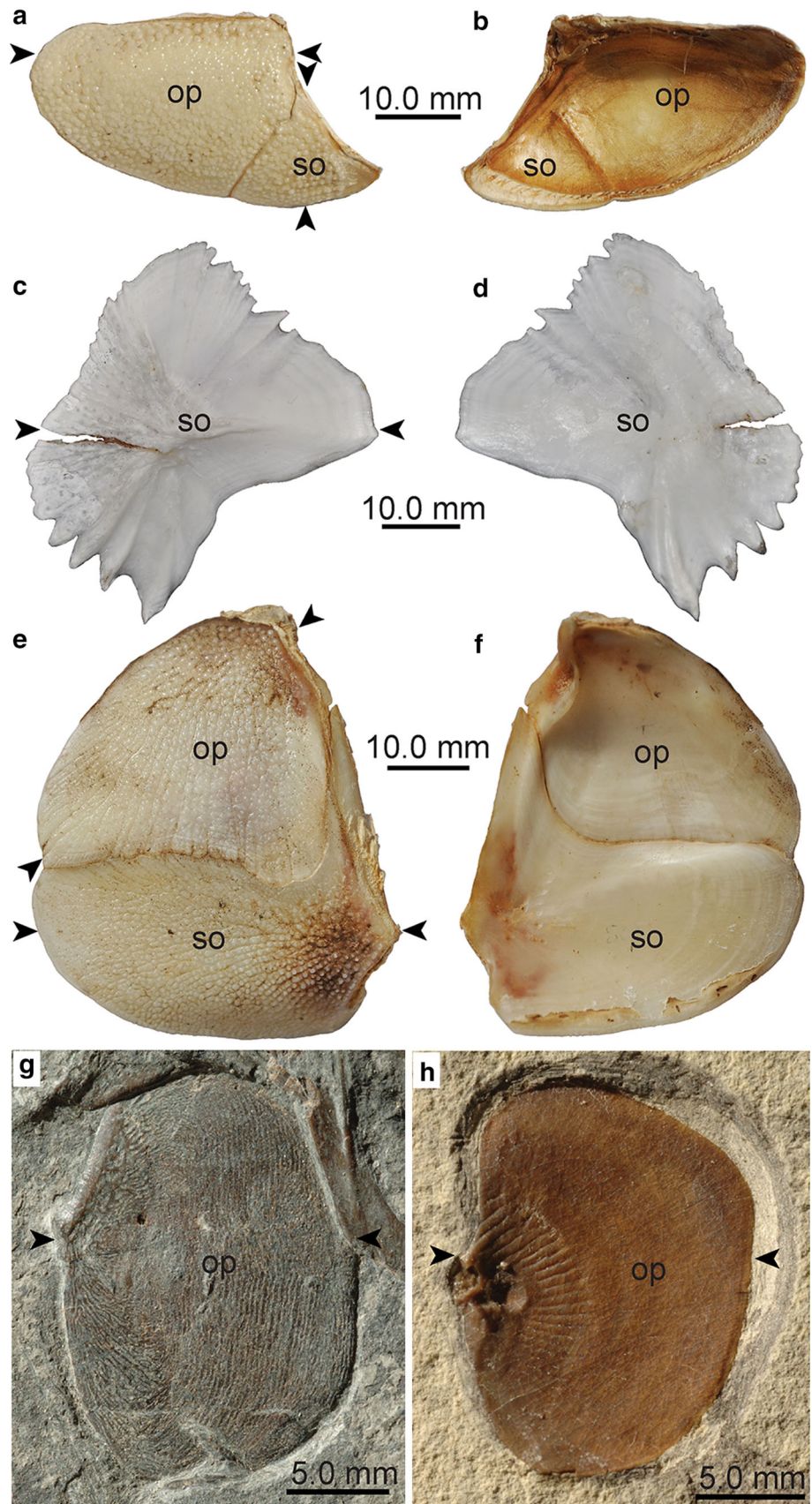
ceratohyal is also paired (a single pair of bones in *Saurichthys* sensu Stensiö 1925) but pertains to the endoskeleton, is of chondral origin and preformed in cartilage (e.g., Patterson 1977; Grande 2010). The term “opercular bones” further refers only to the opercula and subopercula studied.

Results

Outgroups

Both the Siberian sturgeon and spotted gar opercle bones exhibit easily visible growth marks (annuli and growth zones) on their internal surface (Fig. 2), which were best observable in normal transmitted light, or more clearly, in dark-field transmitted light prior to sectioning (=whole

Fig. 2 Opercular elements used in the study. Note that the number of bony elements covering the gills differs among species. **a, c, e, g** Elements in lateral view; **b, d, f, h** in medial (visceral) view. **a, b** *Polypterus bichir* (NMB 2793). **c, d** *Acipenser baerii* (PIMUZ A/I 4309). **e, f** *Lepisosteus oculatus* (PIMUZ A/I 4170). **g** *Saurichthys* sp. (PIMUZ T 2641). **h** *Saurichthys* sp. (PIMUZ T 5736). Pairs of black arrows indicate planes of sectioning. *op* opercle, *so* subopercle



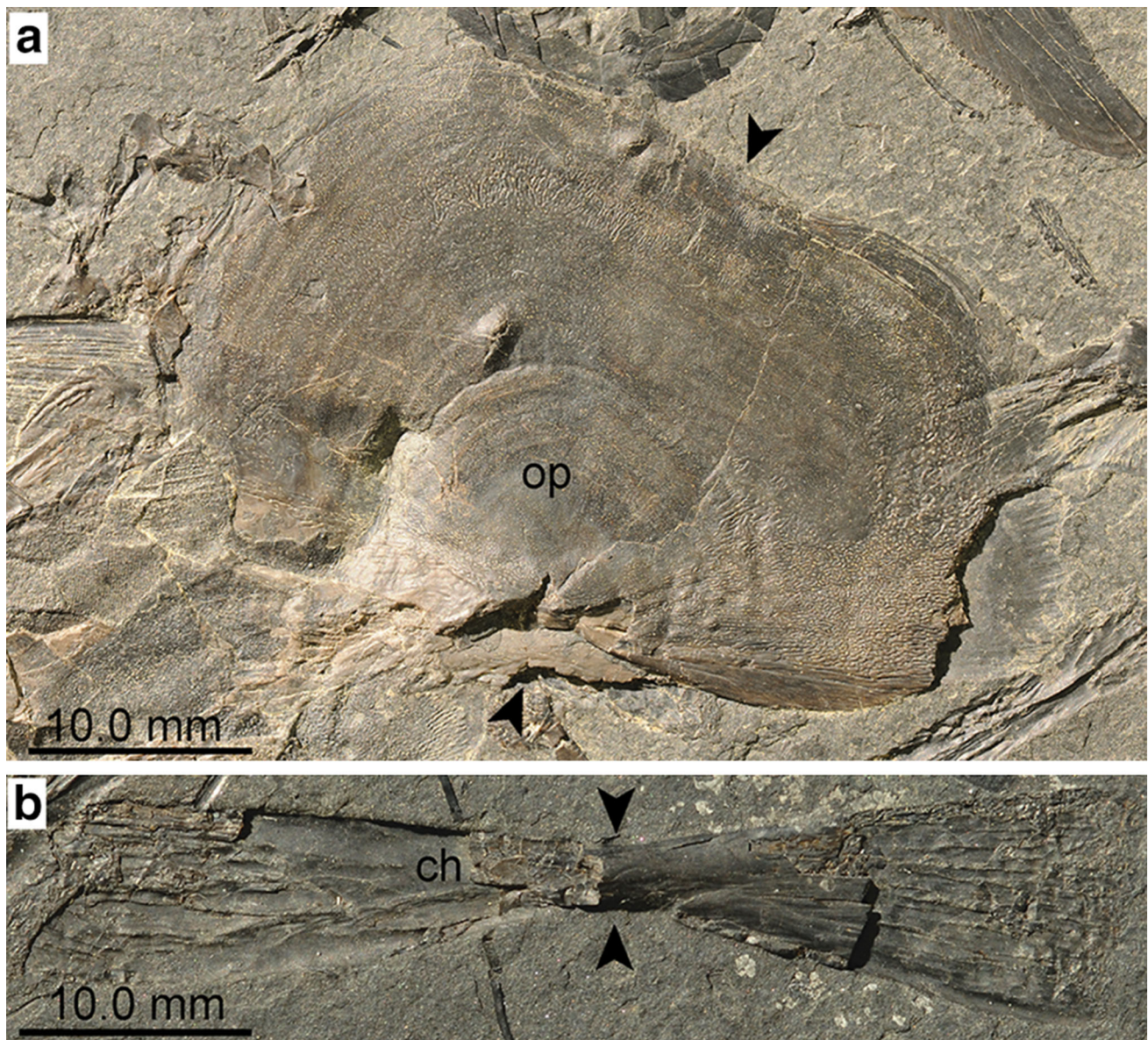


Fig. 3 Largest specimen of *Saurichthys* sp. (PIMUZ T 2138) used in the study. **a** Opercle bone (*op*); **b** ceratohyal (*ch*). Pairs of black arrows indicate planes of sectioning

operculum interpretation). In the Siberian sturgeon their number correlates with the known age of the animals. In the Nile bichir opercle and subopercle no similar growth marks are clearly visible internally, due to a thin layer of connective tissue still being attached to the bone.

Nile bichir (*Polypterus bichir*)

The sectioned bones (subopercle and opercle) can be divided into an internal, lamellate (or stratified) part and an external part which can be vascularized and contains the ornamentation (rows of low tubercular odontode complexes) visible on the external bone surface (Fig. 4a, b).

Larger vascular spaces are restricted to the internal-most parts of the bones, and the external cortex (mostly parallel-fibered bone) is vascularized by a network of branching vascular canals. The internal lamellate part is mostly avascular, but few scattered simple primary vascular canals can occur. Due to the incorporation of larger amounts of Sharpey's fibers, the lamellate tissue is interpreted here as lamellar bone rather than elasmodine (Sire et al. 2009). Similar to the ganoid scales of *Polypterus* (e.g., Meunier 2011; Richter and Smith 1995; Sire 1995), the ornamental tubercular odontodes on both opercular bones are visible in cross section as low-domed ridges or caps of hypermineralized birefringent tissue, ganoine, embedded within the

external cortical bone tissue, often topping or overlapping in a shingle-like fashion (Fig. 4b). The ganoine is pervaded by few thin canaliculi. A thin layer of vascularized tissue, here interpreted as a thin pad of dentine, was found below each ganoine ridge, comparable to the odontocomplex covering the basal plate of the scales (Sire and Akimenko 2004) or the gular plate (Ørvig 1978). The dentine tissue is distinct from the underlying bone tissue in cross-polarized light, as expressed by changes in petrographic extinction patterns and by the lack of bone cell lacunae and canaliculi; however, dentine tubules are not highly conspicuous in any of our thin sections. Scattered large bone cell lacunae with few, but highly conspicuous elongated branching canaliculi are found throughout the remaining bone tissue, whereas canaliculi (canals) of Williamson (Sire and Meunier 1994) are generally absent. Growth marks, here broad growth zones separated by thin annuli followed by lines of arrested growth (LAGs), are best visible in the posterior margins of the opercle and subopercle (Fig. 4c). The growth zones constitute parallel-fibered bone, the annuli are composed of thin layer of slowly deposited lamellar bone, whereas the LAGs are visible as thin slightly hypermineralized lines indicating a growth stop.

Spotted gar (Lepisosteus cf. L. oculatus)

The sectioned bones (subopercle and opercle) are characterized by an external ornamented part and an internal smooth part (Fig. 4d–f). The bone tissue is darker in the area where the external and internal parts of the bone meet in an undulating line and structures are not very clear here, possibly because of superimposition effects. The internal bone tissue is more lamellar in the growth zones, whereas the external growth zones are characterized by a mixture of lamellar and parallel-fibered layers (Fig. 4f). Osteocyte lacunae are flattened and oblong in the lamellar tissue and less so in the parallel-fibered parts. Few branching primary vascular canals pervade the opercular bones, occasionally opening up on the bone surface as foramina. Large vascular cavities are restricted to the thicker, anterior part of the bones carrying the articulation sockets. The tubercular ornamentation of the external bone surface is visible in cross section as ridges and valleys, in which the ridges carry stacks of ganoine layers often intercalating with the surrounding bone tissue. The ganoine layers usually grow in extension with progressing age. As in *Lepisosteus* scales (Sire 1995), there is no indication that dentine resides between the ganoine and the bone tissue. Canaliculi (canals) of Williamson, on the other hand, extend from internal to external throughout the opercular bones as thin tubular structures, often branching terminally, associated with cells of Williamson (Fig. 4g). These co-occur with the osteocytes and their canaliculi, and are interpreted as

serving a nutritional function in the bones (Sire and Meunier 1994). In the basal lepisosteid *Obaichthys* from the Cretaceous of Brasil, scales also show canaliculi of Williamson, but they also retain the plesiomorphic actinopterygian condition with dentine being present between the ganoine layer and the basal plate (Brito et al. 2000).

Growth cycles are clearly visible in the anterior thickened areas (successive parallel growth marks) and towards the posterior margins of the opercular bones of *Lepisosteus*, where growth zones are separated by annuli, followed by a LAG or in some cases double LAGs (Fig. 4f). In the latter, the distal extend of the annuli or LAGs are simple ‘arrow tip’-shaped lines in the central bone area or more complex undulating lines extending from internal to external.

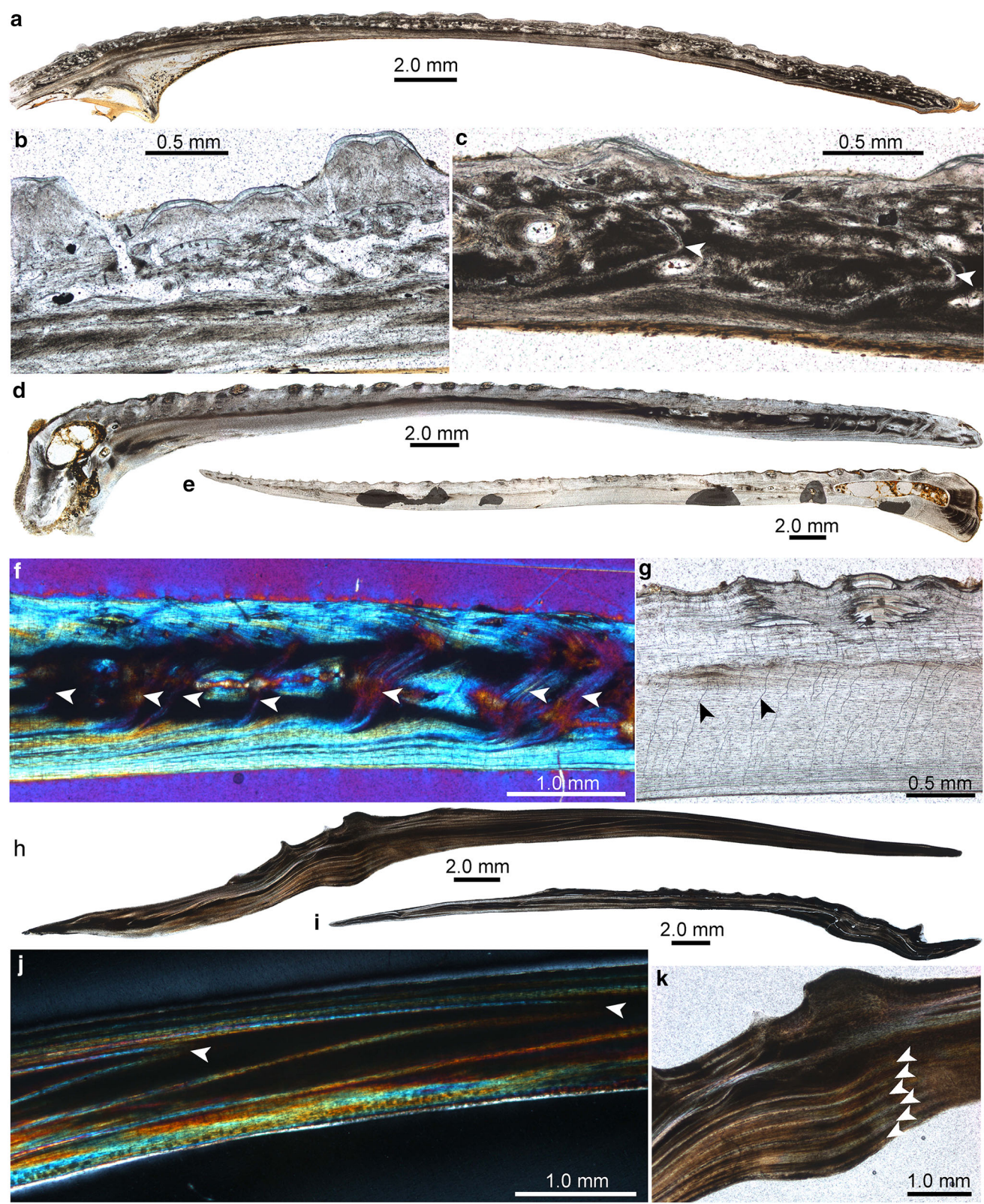
Siberian sturgeon (Acipenser baerii)

The subopercula show already a macroscopically visible alternation of bright and dark bands under incident, or more clearly, in dark-field transmitted light. The sectioned subopercula can be divided into an external and internal part (Fig. 4h, i). Both the internal bone surface and the anterior part of the external bone surface are smooth; with the external ornamentation being restricted to fields of various extensions in the posterior portion of the bones (Fig. 2). In both the external and the internal parts, the tissue is composed mainly of lamellar bone in which the osteocyte lacunae are flattened and oblong. The subopercular bones are largely avascular, although a few scattered and simple primary vascular canals can occur in the thickened posterior parts of the bones (in *A. baerii* the articulation is not situated at the anterior border of the subopercle, but ventrally). The ornamental tubercles consist completely of lamellar bone and lack ganoine ridges. Growth cycles within the subopercula are well visible as darker growth zones and brighter annuli/LAGs (Fig. 4j, k). The latter are best visible both anteriorly as simple arrow tip-shaped lines in the central bone area and internally in the posterior part as stacks of successive sub-parallel lines. In the 3¾-year-old specimens, three widely spaced growth cycles and a significant part of an additional fourth growth zone is visible, whereas in the 6-year-old specimens, six growth cycles plus only the very beginning of a seventh growth zone are visible.

Saurichthys spp. opercula

Opercula

The largest opercle (Fig. 3a) was sampled from specimen PIMUZ T 2138, of which also the ceratohyal bone (Fig. 3b) was analyzed (see below). With up to 2.5 mm at the anterior articulation this bone is the thickest one



◀**Fig. 4** Bone histology of outgroup taxa *Polypterus bichir* (a–c), *Lepisosteus* cf. *L. oculatus* (d–g) and *Acipenser baerii* (h–k). All images in normal transmitted light except **f** in cross-polarized light using lambda compensator and **j** in cross-polarized light. **a** Complete section of opercle (NMB 2793); **b** close-up of external bone surface ornamentation showing ganoine of the odontodes; **c** close-up of posterior part of opercle showing two successive growth marks in the bone tissue (white arrow heads); **d** complete section of opercle (PIMUZ A/I 4170); **e** complete section of subopercle (PIMUZ A/I 4170); **f** close-up of posterior part of opercle showing seven successive growth marks (white arrow heads); **g** close-up of canaliculi (canals) of Williamson (black arrow heads) and external ornamentation with ganoine ridges; **h** complete section of subopercle of 6-year-old female specimen (PIMUZ A/I 4310); **i** complete section of subopercle of 3-year-old female specimen (PIMUZ A/I 4308); **j** close-up of posterior part of subopercle (PIMUZ A/I 4310) showing two successive growth marks in the bone tissue (white arrow heads); **k** close-up of a more anterior section of the subopercle (PIMUZ A/I 4310) showing six successive growth marks as dark growth zones and bright annuli (white arrow heads) in the basal (visceral) part of the bone

sampled, whereas its posterior parts are, with 0.55–0.75 mm, of average thickness. For comparison, the operculum of PIMUZ T 2566 reaches only 1.9 mm at articulation and its more posterior parts range between 0.36 and 1.0 mm, whereas the operculum of PIMUZ T 5501 is 1.05 mm thick at the anterior articulation and between 0.01 and 0.5 mm in its posterior parts.

The opercula consist of an internal, lamellate part and an external, ornamented part (Fig. 5a–d). Both parts exhibit diverging extinction patterns due to differences in bone matrix orientation, with the lamellate part showing birefringence and the ornamental part being non-refracting (possibly woven-fibred matrix). The ornamentation part consists of narrow ridges, often reticulate, which protrude above the remainder of the external bone surface. The ornamental ridges are generally more pronounced in the anterior part of the opercle whereas they are lower and more regular in the posterior part. In section, the ridges are found to be confined to the superficial, ornamental part of the opercle and are not traceable into the lamellate part. Only in some well-preserved specimens (e.g., PIMUZ T 2138; T 2566) a thin layer of tissue can be seen covering the ornamental part of the opercle or filling the ornamental valleys between ridges (Fig. 6). This weakly birefringent thin layer exhibits a fine laminar structure, is acellular and avascular and does not show any extrinsic fibers or tubules. Although it appears structurally similar to elasmodine (Sire et al. 2009), in absence of other parts of an odontode complex in *Saurichthys* opercula, the nature of this thin extra layer remains speculative for the time being.

Deep to the ornamental zone the lamellate part consists of lamellar or parallel-fibred bone, exhibiting cyclical growth marks, i.e., growth zones and annuli/LAGs, both at

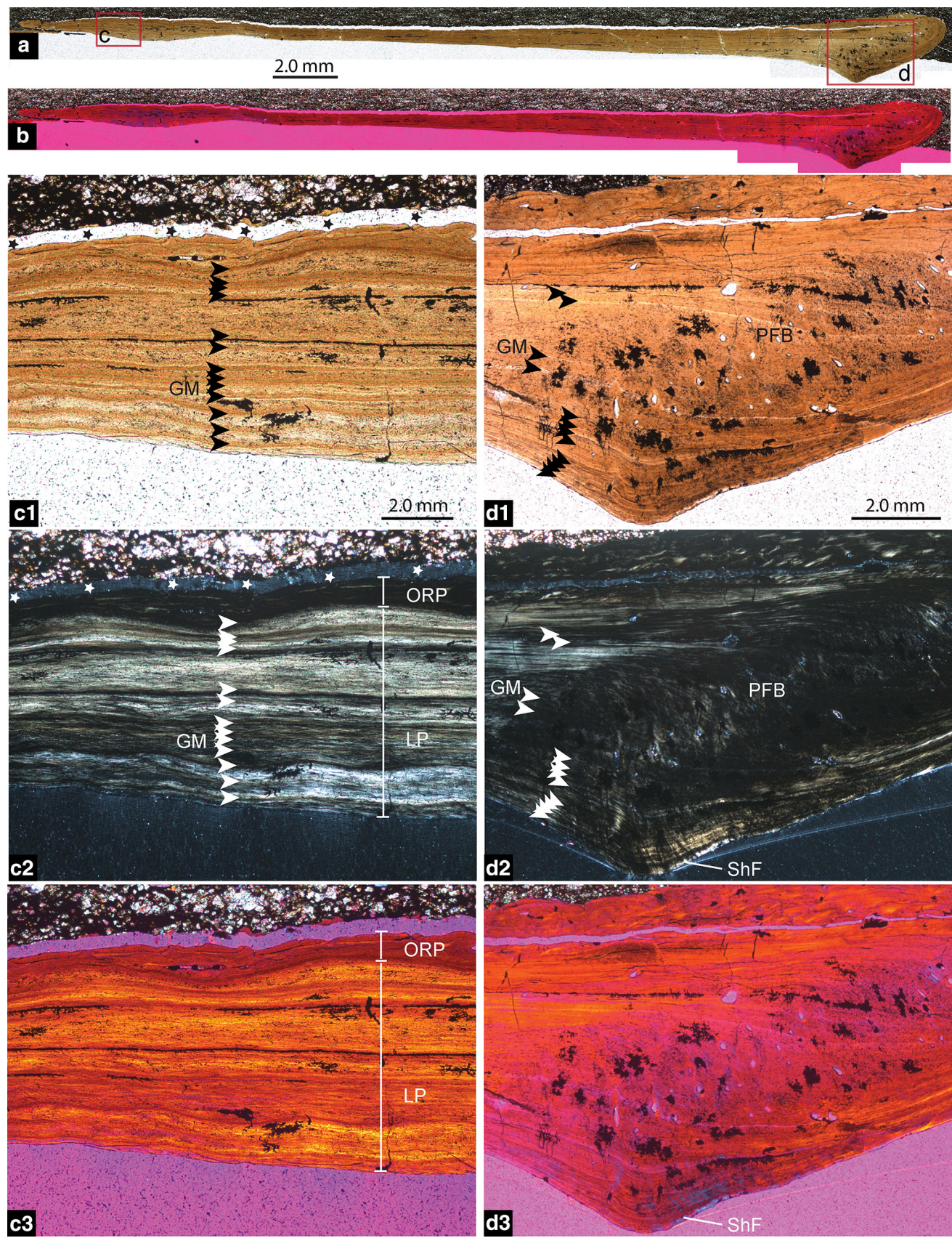
the anterior articulation area and in the flat distal part. The anterior articulation parts of the opercula are vascularized by scattered simple primary vascular canals (Fig. 5d) and occasionally by few scattered secondary osteons, whereas the posterior parts show a reticular network of primary vascular canals and primary osteons, which are confined to the ornamentation zone. A few large scattered foramina may also extend throughout the interior lamellate part as well. Sharpey's fibers are prominent in the anterior articulation part of the opercula, but inconspicuous in the internal lamellate bone of the flat posterior part. Ganoine layers and canaliculi of Williamson are not visible in any of the sections.

Ceratohyal bone

The ceratohyal (PIMUZ T 2138) is roughly kidney-shaped in cross section (Fig. 7a–c), with a well-developed cortex (thickness ranging from 0.5 to 0.8 mm) of avascular parallel-fibred bone surrounding a slightly compacted internal cavity (long axis: 1.55 mm, short axis: ca. 0.3 mm). The latter is partitioned by few internal bone trabeculae consisting of secondary endochondral lamellar bone. The border of the cavity is mostly lined with a layer of endochondral lamellar bone, but it locally shows signs of subsequent remodeling in the form of Howship's lacunae. Secondary osteons are absent. Two sets of Sharpey's fibers are recognizable extending throughout the cortex in the convex part of the bone. Cell lacunae are sparsely distributed all over the cortex, slightly oblong in the lamellar bone, and carry inconspicuous canaliculi. A minimum of 14 growth marks (zones, annuli or LAGs) were counted in the ceratohyal. Given the size of the interior cavity, the signs of cortical remodeling, as well as based on the overall thickness of the growth zones visible, the internal-most growth record (one or two growth cycles, three at the most) is interpreted to have been erased.

Discussion

It is generally assumed that annuli in the fish skeleton reflect seasonal periodicity (Campana 2001; Panfili et al. 2002), and consequently annuli were reported to be lacking or blurred in fishes raised in aquaculture (Campana 2001), as well as in tropical fishes due to lack of strong seasonality (Devries and Frie 1996, but see Brusher and Schull 2009; Fowler 2009; Lou 1992; Morales-Nin 1989 for opposing interpretations). Age estimates by any method and use of any structure have to be validated by comparison with the true age of fish and accuracy, the closeness of the age estimate to the true value, has to be distinguished from precision, the reproducibility of repeated measurements on



◀**Fig. 5** Bone histology of *Saurichthys* opercle PIMUZ T 2566. Images **a**, **c1**, **d1** in normal transmitted, **c2**, **d2** in cross-polarized and **b**, **c3**, **d3** in cross-polarized light using lambda compensator. **a**, **b** Cross section of whole opercle (anterior towards the right of image). Position of close-ups shown in **b** and **c** are indicated by red squares; **c1**–**c3** close-up of flat posterior part of opercle. Note that tips of ornamental ridges (indicated by black and white asterisks in **c1** and **c2**) were separated from the rest of the opercle during thin-sectioning; **d1**–**d3** close-up of anterior articulation part of opercle. Note Sharpey's fibers inserting internally into the bone matrix. Growth marks (annuli/LAGs between growth zones) are visible throughout the bone tissue as indicated by black or white arrow heads in **c** and **d**. *GM* growth marks, *LP* lamellate part of opercle, *ORP* ornamented part of opercle, *PFB* parallel-fibered bone, *ShF* Sharpey's fibers

a given structure (Campana 2001). Precision, not only accuracy of age estimates, is highest using otoliths in most cases, whereas equally good data based on opercula were found only in few specific cases (Quist et al. 2007).

In acipenserids, whole subopercula were used for age estimation in two species, in the white sturgeon *Acipenser transmontanus* (Brennan and Cailliet 1989) and in the shovelnose sturgeon *Scaphirhynchus platyrhynchus* (Jackson et al. 2007). In both cases, however, counting annuli was difficult, leading to biases in precision, and results are, therefore, of limited use. Furthermore, applying histological techniques was found to be technically difficult and they failed to provide clear annual growth data (Jackson et al. 2007). In *Acipenser sturio*, the European sturgeon, the deposition of thin translucent rings in pectoral fin rays during the winter period (and larger opaque rings during the rest of the year) could be demonstrated (Jatteau et al. 2011). The difference is usually attributed to low water temperature and scarce food resources during winter (Cochner et al. 1985; Jatteau et al. 2011), but there are

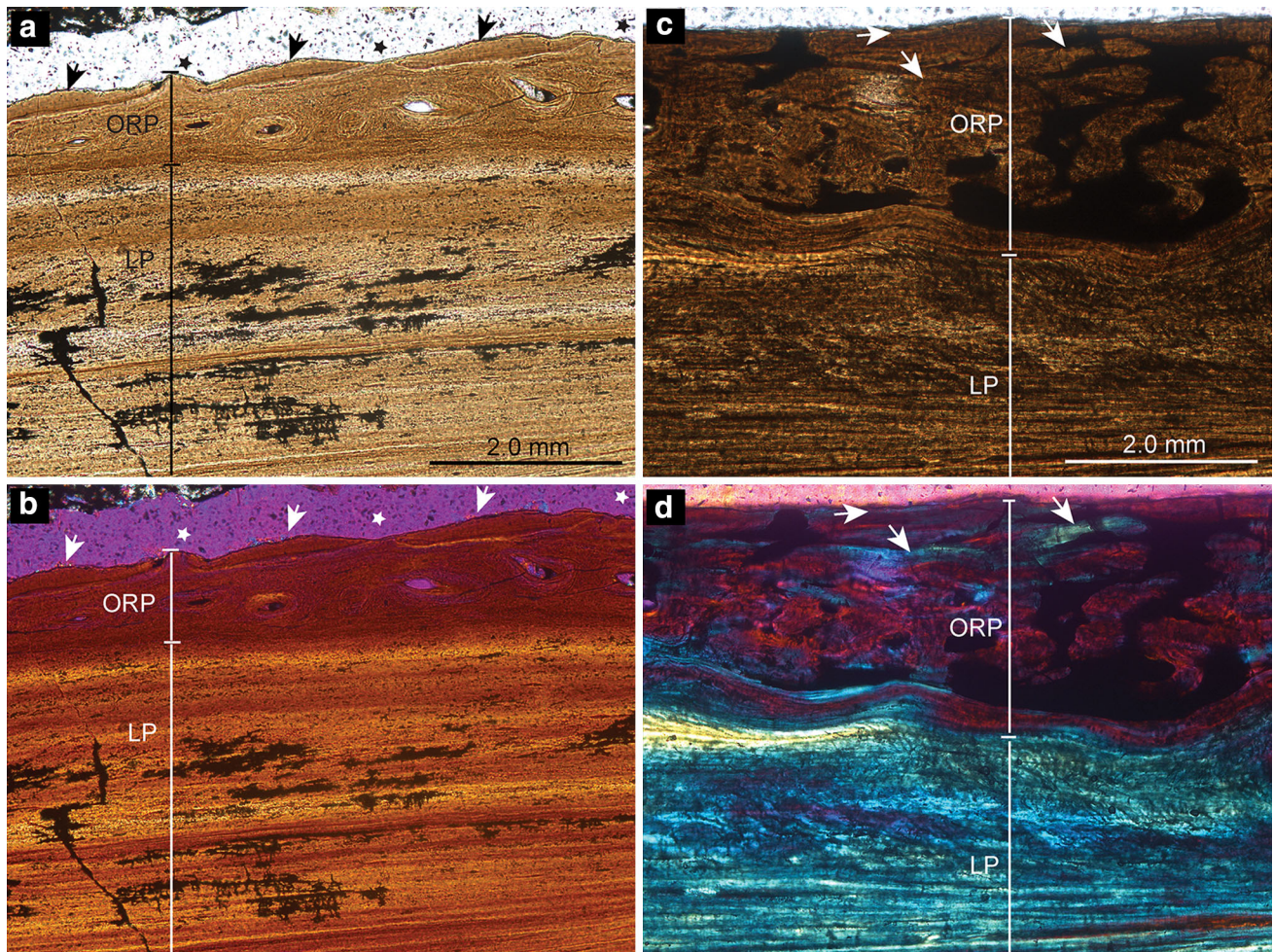
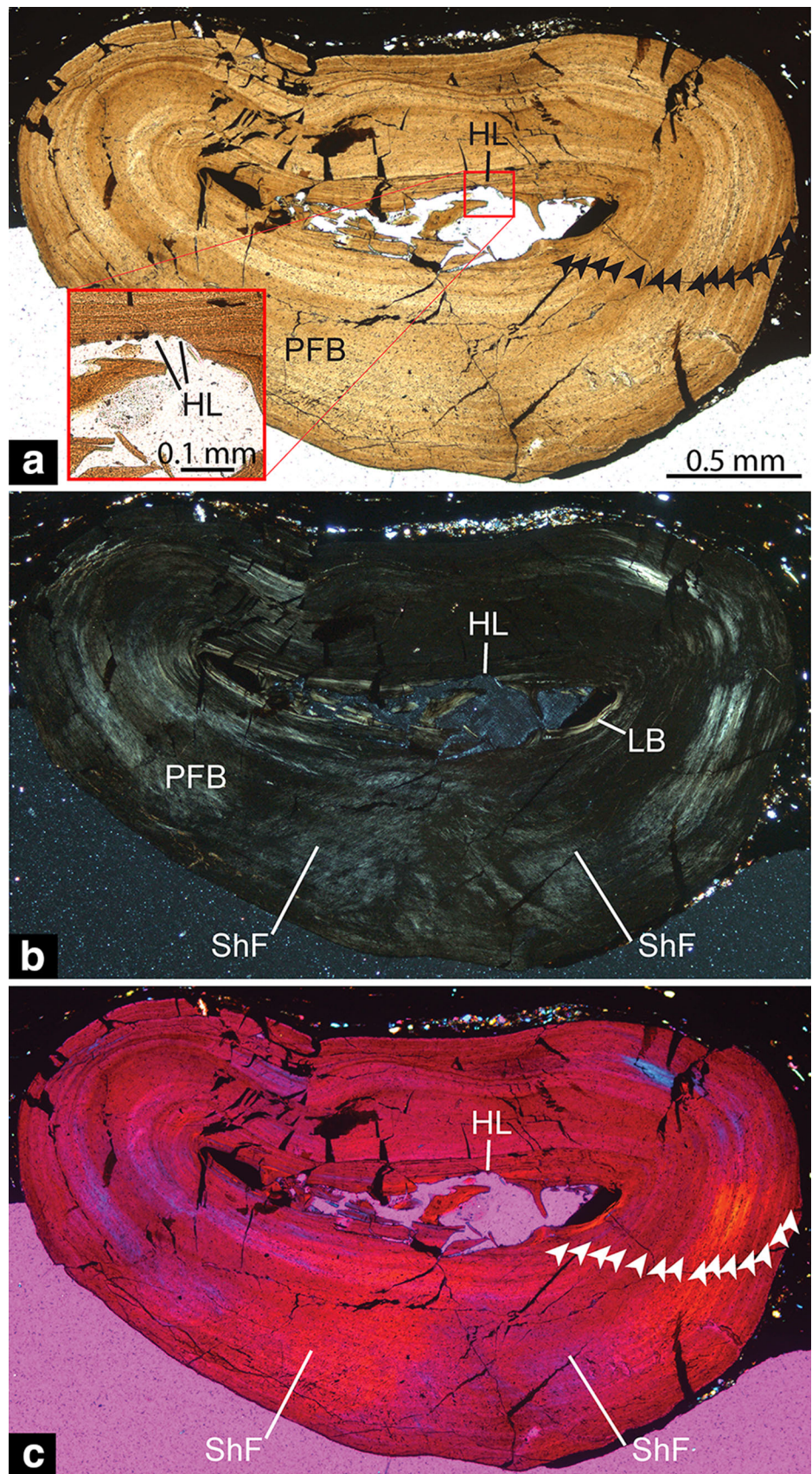


Fig. 6 Bone histology of *Saurichthys* opercula PIMUZ T 2566 (**a**, **b**) and T 2138 (**c**, **d**). Images **a**, **c** in normal transmitted, **b**, **d** in cross-polarized light using lambda compensator. **a**, **b** Close-up of thin external ornamented part and underlying lamellate part of opercle. Note broken-off ornamental ridges in cross section (indicated by black and white asterisks) and adjacent thin layers of only slightly

birefringent tissue (black and white arrows); **c**, **d** close-up of well vascularized external ornamented part and underlying lamellate part of opercle. Note overall non-refractive of the ornamental part with the exception of few thin superficial layers (marked by white arrows). *LP* lamellate part of opercle, *ORP* ornamented part of opercle

Fig. 7 Bone histology of central shaft area of ceratohyal of *Saurichthys* sp. (PIMUZ T 2138). Image **a** in normal transmitted, **b** in cross-polarized, and **c** in cross-polarized light using lambda compensator. The *inset* in **a** shows the area where Howship's lacunae were identified. Note that specimen has been slightly crushed and, therefore, the outline of the bone and its medullary cavity would have been more oval. Fourteen growth marks (as indicated by *black* and *white* arrow heads) are well visible in the section. The medullary cavity was at least partly lined with endosteal lamellar bone and comprised few internal trabeculae. *HL* Howship's lacuna, *LB* lamellar bone, *PFB* parallel-fibered bone, *ShF* Sharpey's fibers



indications that temperature alone might be responsible (Bruch et al. 2009). In the present study, the *Acipenser baerii* specimens could be aged with confidence and indeed the laid down growth cycles correspond well with the actual age of the animals in both age classes sampled. Despite being raised under more constant environmental conditions in their latter part of life (i.e., under more constant annual temperatures), the older age group specimens still provided well-visible growth cycles throughout life, indicating that temperature is not the only variable affecting bone deposition and incremental growth in these animals. In the opercle bones of the Nile bichir and the spotted gar, growth cycles are also relatively easy to determine, but the exact age at death of the animals is not known, therefore, precluding a cross-validation.

Proposed morphogenetic model of histogenesis in *Saurichthys* opercula

Growth marks were visible in all *Saurichthys* specimens, but these marks could not be counted to give reliable age estimates. Even the largest specimen sampled, PIMUZ T 2138, which has a partly thicker opercle compared to the other *Saurichthys* opercula, nevertheless lacks clear growth marks that could be compared to the 14 growth cycles found in the sectioned ceratohyal element of the same specimen.

As such, structures indicating the distal extend of annuli (Fig. 8a) as described for longnose sucker (Perry and Casselman 2012) and which herein were also found in all three outgroup taxa (the conspicuous arrow tip-shaped distal extend of growth cycles), could not be identified in *Saurichthys* opercula. This is mainly due to three factors:

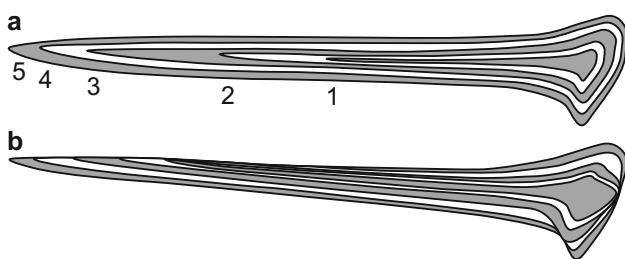


Fig. 8 Schematic drawing of the histomorphogenic model of opercular growth. **a** Opercular growth as was found in the outgroup species *Polypterus bichir*, *Acipenser baerii*, and *Lepisosteus* cf. *L. oculatus*. This model is in accordance with previously published results of opercular growth in other fish species (e.g., Perry and Casselman 2012). Numbers indicate hypothetical successive growth stages countable in the cross sections; **b** derived growth model of *Saurichthys* opercula. Note that the counting of successive growth stages is hampered by preferential growth so that growth marks cannot be followed throughout the sections. In the opercular bones, ornamentation and remodeling processes further decrease identification of growth marks in the bone tissue

(1) *Saurichthys* opercula are extremely thin (the bones are estimated to be thin already in life and maybe even further compressed during fossilization) compared to the opercular bones of the extant outgroups, thus impeding the recognition of clear growth marks in many parts of the bones. (2) Growth lines generally extend from rostro-medial to caudo-lateral instead of extending more or less sub-parallel to the bone surface. This indicates that growth in *Saurichthys* opercula occurred mainly on the medial (internal) surface and on the posterior border (Fig. 8b). (3) Some variation was observable in opercular thickness, overall amount and distribution of vascularization, as well as in patterning and morphology of ornamental ridges and adjacent valleys, in many cases further obscuring growth marks in the bone tissue. At the moment, the question remains open whether these factors are linked to differences among species, taphonomy, diagenesis, or to sex or age differences of individuals.

Surface ornamentation on *Saurichthys* opercula

The external surfaces of *Saurichthys* opercula show numerous low ridges and grooves of about 0.2–0.3 mm distance. However, these are not strictly concentric and show intersections and ramifications (Figs. 1a, 2g). As indicated by the thin sections (Figs. 5, 6), the above-mentioned ridges on the external surface are excrescences of a few externally situated growth layers and not intersections of internal growth marks with the external bone surface. Thus, the protuberances and consequently the ridges on the surface aspect are not indicators of growth.

On the internal surface of the opercula, broad concentric bands or wrinkles could be observed (Fig. 2h) but failure to cross-correlate those with the internal growth marks seen in thin sections sheds doubt on their interpretation as annuli or growth zones as well. There is no periodicity of growth lines in the sections that would correspond to the bands on the internal surface of opercula. Whether the internal concentric bands serve a mechanical purpose in stiffening the extremely thin opercula, or whether they are of taphonomic origin (e.g., due to shrinking, dehydration and deformation processes of the tissue during fossilization) remains speculative.

Conclusions

Growth marks are visible in all *Saurichthys* opercula studied. Although these bones are very prominent and relatively easy to section, individual age data could not be gathered from these, due to difficulties of reliably identifying all age-related structures and because of morphogenetic differences in histogenesis between the Triassic fish

and the modern non-teleost actinopterygian outgroups. Furthermore, both the external and internal bone surface patterns on the *Saurichthys* opercula could be shown to be either ornamental or structural/taphonomic in nature and not related to cyclical growth of the animal. On the other hand, growth cycles were well visible and more easily countable in the ceratohyal, a bone with an ovoid shaft cross section, making it a suitable candidate for further skeletochronological studies. Consequently, we consider age estimates based on opercula alone unreliable in *Saurichthys*, and advise focusing on cylindrical elements for future studies interested in the life history of these Triassic predatory fishes.

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